

Claims

5 1. A process for the preparation of autologous cells for the prevention and/or treatment of diseases associated with disturbed self-tolerance in a patient, characterised in that

10 a) monocytes are isolated from the blood of the patient to whom the cells are to be administered;

b) the monocytes are multiplied in a suitable culture medium which contains the cellular growth factor M-CSF;

15 c) the monocytes are cultivated simultaneously with or following step b) in a culture medium containing γ -IFN; and

20 d) the cells formed in step c) are obtained by separating the cells from the culture medium.

2. A process according to claim 1 characterised in that the monocytes are of human origin.

25 3. A process according to claims 1 or 2 characterised in that the monocytes are isolated from the blood in such a manner that next to the monocytes also lymphocytes are present in an amount of at least 10% by reference to the total cell number in the isolate.

30 4. A process according to claims 1 to 3, characterised in that the cells formed in step c) or obtained in step d)

are selected by binding to the antibody produced by the hybridoma cell line DSM ACC2542.

5. A process according to claims 1 to 4, characterised in that among the cells formed in step c) or obtained in step d) of claim 1 or obtained in the selection step according to claim 4, those cells are selected which co-express the antigens CD3 and CD14 on their cell surface.
- 10 6. A process according to claims 1 to 5, characterised in that the M-CSF concentration in the culture medium is 1 to 20 µg/l.
- 15 7. A process according to claims 1 to 6, characterised in that, subsequent to step b) the monocytes are cultivated for 24 to 72 hours in a culture medium containing γ-IFN, the cultivation in the presence of γ-IFN beginning 3 to 6 days after the beginning of cultivation step b).
- 20 8. A process according to claim 7, characterised in that the γ-IFN concentration in the culture medium is 0.1 to 20 ng/ml.
- 25 9. A process according to claims 1 to 8 characterised in that the total cultivation period in steps b) and c) is 4 to 8 days.
- 30 10. A process according to claims 1 to 9 characterised in that subsequent to step d) of claim 1, or subsequent to the selection steps according to claims 4 and 5, the cells are suspended in a suitable cell culture medium or in a PBS or NaCl solution.

11. A process according to claims 1 to 10 characterised in that the cells are suspended in a freezing medium and are subsequently deep frozen.
- . 5 12. A process according to claim 11 characterised in that the freezing medium comprises fetal calf serum (FCS) or human ABO compatible serum and DMSO.
- 10 13. Autologous cells for the prevention and/or treatment of diseases associated with disturbed self-tolerance in patient, obtainable by any of the processes according to claims 1 to 12.
- 15 14. Cells according to claim 13 characterised in that they co-express the antigens CD3 and CD14 on their cell surface.
- . 15. Cells according to claims 13 or 14 characterised in that they are of human origin.
- 20 16. Cell preparation containing the cells according to claims 13 to 15 in a suitable medium.
- 25 17. Pharmaceutical composition containing autologous cells of monocytic origin for the prevention and/or the treatment of diseases associated with disturbed self-tolerance in a patient.
- 30 18. Pharmaceutical composition containing the cells according to claims 13 to 15 or the cell preparation according to claim 16.

19. Pharmaceutical composition according to claims 17 and 18 for the prevention and/or the treatment of autoimmune diseases.

5 20. Pharmaceutical composition according to claims 17 and 18 for the prevention and/or the treatment of allergies.

10 21. Use of the cells according to claims 13 to 15 or the cell preparation according to claim 16 for manufacturing a pharmaceutical composition for the prevention and/or treatment of diseases associated with disturbed self-tolerance.

15 22. Use according to claim 21 for the prevention and/or treatment of autoimmune diseases.

20 23. Use of claim 22, characterised in that the autoimmune disease is one or more of the diseases selected from rheumatic diseases with autoimmune features, diabetes mellitus, autoimmune diseases of the blood and blood vessels, autoimmune diseases of the liver, autoimmune diseases of the thyroid, autoimmune diseases of the central nervous system, and bullous skin diseases.

25 24. Use according to claim 21 for the prevention and/or treatment of allergies.

30 25. Use according to claim 24, characterised in that the allergy is selected from allergies induced by non-self proteins, organic substances and/or inorganic substances.

26. Use according to claim 25, characterised in that the allergy is selected from hayfever and/or allergies induced

by drugs, chemicals, viruses, bacteria, fungi, food components, metals, gases, cat skin scale and/or animal hair.

- 5 27. The use of self-tolerance inducing cells according to claims 13 to 15 or the cell preparation of claim 16 for *in vitro* generating and/or propagating autologous regulatory T-lymphocytes.
- 10 28. The use according to claim 27, wherein the regulatory T-lymphocytes co-express the antigens CD4 and CD25 on their cell surface.
- 15 29. A process for the generation and/or propagation of autologous regulatory T-lymphocytes, characterised in that
 - 20 a) self-tolerance inducing cells according to claims 13 to 15 or a cell preparation according to claim 16 are co-cultivated with an autologous T-lymphocyte preparation, and
 - 25 b) the regulatory T-lymphocytes are optionally obtained from the culture medium.
- 30 30. A process according to claim 29, characterised in that the regulatory T-lymphocytes co-express the antigens CD4 and CD25 on their cell surface.
- 30 31. A process according to claims 29 or 30, characterised in that the regulatory T-lymphocytes are obtained from the culture medium by FACS sorting.

32. Regulatory T-lymphocytes obtainable by the process of claims 29 to 31.
33. The use of the antibodies produced by the hybridoma cell line DSM ACC2542 for the detection and/or selection of autologous cells suitable for the prevention and/or treatment of diseases associated with disturbed self-tolerance in a patient.
5
34. A method for the prevention and/or treatment of diseases associated with disturbed self-tolerance in a patient, characterised in that a pharmaceutically effective amount of the autologous cells according to claims 13 to 15 or the autologous cell preparation according to claim 16 is administered to the patient.
10
35. The method according to claim 34, for the prevention and/or treatment of autoimmune diseases.
15
36. The method according to claim 35, wherein the autoimmune disease is one or more of the diseases selected from rheumatic diseases with autoimmune features, diabetes mellitus, autoimmune diseases of the blood and blood vessels, autoimmune diseases of the liver, autoimmune diseases of the thyroid, autoimmune diseases of the central nervous system, and bullous skin diseases.
20
37. The method of claim 34 for the prevention and/or treatment of allergies.
25
38. The method of claim 37, wherein the allergy is selected from allergies induced by non-self proteins, organic substances and/or inorganic substances.
30

39. The method of claim 38, wherein the allergy is selected from hayfever and/or allergies induced by drugs, chemicals, viruses, bacteria, fungi, food components, metals, gases, animal skin scale, hair and/or animal excreta.